REMARKS

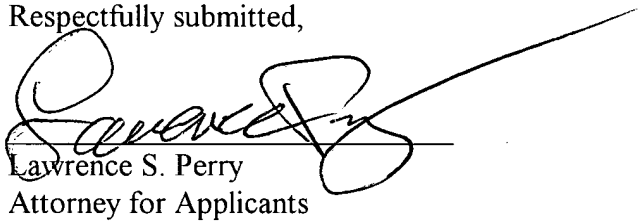
Claims 5, 13, 14, 16-19, 23, 25, 29-34, 37-40, 42-44 and 46 have been amended to correct their dependency and conformity with accepted U.S. practice.

No new matter has been added.

Entry hereof is earnestly solicited.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

5. (Amended) A DNA selected from the group consisting of:

(a) DNA coding for the polypeptide described in [any one of] claims 1 [to 4] or 2,

(b) DNA having the nucleotide sequence of 402 to 1331 in the nucleotide sequence represented by SEQ ID NO: 2,

(c) DNA having the nucleotide sequence of 492 to 1331 in the nucleotide sequence represented by SEQ ID NO: 2, and

(d) DNA hybridizing under stringent conditions with the DNA described in any of (a) to (c) and coding for a polypeptide having b1,3-galactosyltransferase activity capable of synthesizing Galb1-3GlcNAc structure.

13. (Amended) A process for producing the polypeptide described in [any one of] claims 1 [to 4] or 2, which comprises culturing a transformant harboring a recombinant DNA prepared by integrating DNA coding for the polypeptide [of any one of claims 1 to 4] into a vector in a medium to thereby form and accumulate said polypeptide in the culture, and collecting said polypeptide from said culture.

14. (Amended) A process for producing the polypeptide described in [any one of] claims 1 [to 4] or 2, which comprises breeding a non-human transgenic animal harboring a recombinant DNA prepared by integrating DNA coding for the polypeptide [of any one of claims 1 to 4] into a vector to thereby form and accumulate said polypeptide in said animal, and collecting.

16. (Amended) A process for producing the polypeptide described in [any one of] claims 1 [to 4] or 2, which comprises culturing a transgenic plant harboring a recombinant DNA prepared by integrating DNA coding for the polypeptide [of any one of claims 1 to 4] into a vector to thereby form and accumulate said polypeptide in said plant, and collecting said polypeptide from said plant.

17. (Amended) A process for producing the polypeptide described in [any one of] claims 1 [to 4] or 2, which comprises synthesizing the polypeptide [of any one of claims 1 to 4] in an in vitro transcription-translation system using DNA coding for said polypeptide.

18. (Amended) A process for producing a reaction product having galactose, which comprises using [the] a polypeptide having β1,3- galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer

cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure [of any one of claims 1 to 4] as an enzyme source, and allowing

(a) said enzyme source,

(b) an acceptor substrate selected from the group consisting of:

i) N-acetylglucosamine (GlcNAc),

ii) an oligosaccharide having N-acetylglucosamine residue at the non-reducing terminus thereof, and

iii) a complex carbohydrate having N-acetylglucosamine residue at the non-reducing terminus thereof, and

(c) uridine-5'-diphosphate galactose to be present in an aqueous medium to thereby form and accumulate said reaction product in the aqueous medium, and collecting said reaction product from said aqueous medium, wherein the galactose is transferred via

β1,3-linkage to N-acetylglucosamine or N-acetylglucosamine residue of said acceptor substrate.

19. (Amended) A process for producing a reaction product having galactose, which comprises using [the] a polypeptide [of any of claims 1 to 4] having β1,3-galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure as an enzyme source, and allowing

(a) said enzyme source,

(b) an acceptor substrate selected from the group consisting of:

i) glucose,

ii) an oligosaccharide having glucose residue at the non-reducing terminus thereof, and

iii) a complex carbohydrate having glucose residue at the non-reducing terminus thereof, and

(c) uridine-5'-diphosphate galactose to be present in an aqueous medium to thereby form and accumulate said reaction product in the aqueous medium, and collecting said reaction product from said aqueous medium, wherein the galactose is transferred via β1,3-linkage to glucose or glucose residue of said acceptor substrate.

23. (Amended) A process according to any [one] of claims 18 to 20 [22] wherein the complex carbohydrate is a complex carbohydrate selected from the group consisting of a glycoprotein, a glycolipid, a proteoglycan, a glycopeptide, a lipopolysaccharide, a peptidoglycan and a glycoside which is a steroid compound with a sugar chain.

25. (Amended) A method for determining the expression level of a gene encoding the polypeptide of [any one of] claims 1 [to 4] or 2, which comprises hybridization using DNA coding for said polypeptide or a fragment of said DNA.

29. (Amended) A method for determining the expression level of a gene encoding [the] a polypeptide [of any one of claims 1 to 4] having β1,3-galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain,

present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure, which comprises polymerase chain reaction using the [oligonucleotide] DNA of [any one of] claim[s] 26 [to 28].

30. (Amended) A method for detecting cancers and cancer metastasis, which comprises using the method of claim [25 or] 29.

31. (Amended) A method for inhibiting transcription of DNA coding a [for the] polypeptide [of any one of claims 1 to 4] having β1,3- galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure or translation of its corresponding mRNA, which comprises using a DNA [selected from DNAs] of claim[s 5 and] 26 [to 28] and or a DNA[s] having a nucleotide sequence represented by SEQ ID NO: 2 or 3.

32. (Amended) An antibody recognizing the polypeptide of [any one of] claims 1 [to 4] or 2.

33. (Amended) A method for immunological detection of [the] a polypeptide [of any one of claims 1 to 4] having β1,3- galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure, which comprises using the antibody of claim 32.

34. (Amended) An immunohistostaining method, which comprises detecting [the] a polypeptide of [any one of claims 1 to 4] having β1,3-galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure by using the antibody of claim 32.

37. (Amended) A method for screening a compound varying the activity of the polypeptide of [any one of] claims 1 [to 4] or 2, which comprises contacting said polypeptide with a test sample.

38. (Amended) A method for screening a compound varying the expression of a gene coding for the polypeptide of [any one of] claims 1 [to 4] or 2, which comprises contacting cells expressing said polypeptide with a test sample and determining the content of sialyl-Lewis a sugar chain, Lewis a sugar chain, Lewis b sugar chain or sialyl-Lewis c sugar chain by use of anti-sialyl-Lewis a antibody, anti-Lewis a antibody, anti-Lewis b antibody or anti-sialyl-Lewis c antibody.

39. (Amended) A method for screening a compound varying the expression of a gene coding for [the] a polypeptide [of any one of claims 1 to 4] having β1,3- galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure, which comprises contacting cells expressing said polypeptide with a test sample and determining the content of said polypeptide by use of the antibody of claim 32.

40. (Amended) A promoter DNA governing transcription of a gene coding for [the] a polypeptide [described in any one of claims 1 to 4] having β1,3-galactosyltransferase activity involved in the synthesis of sialyl-Lewis a sugar chain, present in colon cancer cells expressing sialyl-Lewis a sugar chain, or a polypeptide which is selected from the group consisting of:

(a) a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 1,

(b) a polypeptide containing the amino acid sequence of 31 to 310 in the amino acid sequence represented by SEQ ID NO: 1, and

(c) a polypeptide consisting of an amino acid sequence where in the amino acid sequence of the polypeptide (a) or (b), one or more amino acids have been deleted, replaced or added and having β1,3-galactosyltransferase activity capable of synthesizing Galβ1-3GlcNAc structure .

42. (Amended) A promoter DNA according to claim [40 or] 41, which is a human- or mouse-derived promoter DNA.

43. (Amended) A promoter DNA according to [any one of] claims 40 [to 42] or 41, which comprises a 50- to 5000-bp consecutive nucleotide DNA sequence in the nucleotide sequence of 1 to 5000 in the nucleotide sequence represented by SEQ ID NO: 3.

44. (Amended) A method for screening a compound varying the efficiency of transcription by the promoter DNA of [any one of] claims 40 [to 43] or 41, which comprises transforming animal cells with a plasmid containing said promoter DNA and a reporter gene ligated downstream of said promoter DNA, then contacting the transformant with a test sample, and determining the content of a translation product of said reporter gene.

46. (Amended) A knockout non-human animal wherein a DNA coding for the polypeptide of [any one of] claims 1 [to 4] or 2 is rendered defective or mutated.

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO SPECIFICATION

The paragraph at page 4, lines 8-13 have been amended as follows:

The object of the present invention is to provide a pharmaceutical preparation for anti-inflammations, anti-infections or inhibition of cancer metastasis, foods such as dairy products, a method of improving proteins, and a method for diagnosis of diseases such as cancers, by utilizing a novel polypeptide having β1,3-galactosyltransferase activity.

The paragraph starting at page10, line 36 and ending at page 11, line 2 has been amended as follows:

25. A method for determining the expression level of a gene encoding the polypeptide of any one of items 1 to 4, which comprises hybridization using DNA coding for said polypeptide or a fragment of said DNA.

The paragraph at page 11, lines 10-27 have been amended as follows:

27. A[n oligonucleotide] DNA according to item 26 wherein the oligonucleotide derivative is selected from the group consisting of an oligonucleotide derivative in which the phosphodiester bond is converted into a phosphorothioate bond, an oligonucleotide derivative in which the phosphodiester bond is converted into an N3'-P5'-

phosphoamidate bond, an oligonucleotide derivative in which the ribose and the phosphodiester bond are converted into a peptide-nucleic acid bond, an oligonucleotide derivative in which the uracil is replaced by a C-5 propynyluracil, an oligonucleotide derivative in which the uracil is replaced by a C-5 thiazolyluracil, an oligonucleotide derivative in which the cytosine is replaced by a C-5 propynylcytosine, an oligonucleotide derivative in which the cytosine is replaced by a phenoxazine-modified cytosine, an oligonucleotide derivative in which the ribose is replaced by a 2'-O-propylribose, and an oligonucleotide derivative in which the ribose is replaced by a 2'-methoxyethoxyribose.

The paragraph at page 11, lines 30-33 have been amended as follows:

29. A method for determining the expression level of a gene encoding the polypeptide of any one of items 1 to 4, which comprises polymerase chain reaction using the [oligonucleotide] DNA of any one of items 26 to 28.

The paragraph at page 17, lines 30-33 have been amended as follows:

The expression vector into which the cDNA has been integrated is introduced into animal cells capable of [selecting] expressing the objective cDNA, to give transformed cells.

The paragraph at page 22, lines 7-26 have been amended as follows:

The expression vector includes, e.g., pBTrp2, pBTac1, pBTac2 (which all are commercially available from Boehringer Mannheim), pKK233-2 (a product of Pharmacia), pSE280 (a product of Invitrogen), pGEMEX-1 (a product of Promega), pQE-8 (a product of QIAGEN), pKYP10 (Japanese Published Unexamined Patent Application No. 110600/83), pKYP200 [Agric. Biol. Chem., 48, 669 (1984)], pLSA1 [Agric. Biol. Chem., 53, 277 (1989)], pGEL1 [Proc. Natl. Acad. Sci., USA, 82, 4306 (1985)], pBluescript II SK+ (a product of Stratagene), pBluescript II SK(-) (a product of Stratagene), pTrs30 (FERM BP-5407), pTrs32 (FERM BP-5408), pGHA2 (FERM BP-400), pGKA2 **[**(FERM B-6798)**]** **(FERM BP-6798)**, pTerm2 (Japanese Published Unexamined Patent Application No. 22979/91, US 4686191, US 4939094, US 5160735), pEG400 [J. Bacteriol., 172, 2392 (1990)], pGEX (a product of Pharmacia), pET system (a product of Novagen), pSupex, pUB110, pTP5, pC194, pTrxFus (a product of Invitrogen), pMAL-c2 (a product of New England Biolabs), pUC19 [Gene, 33, 103 (1985)], pSTV28 (a product of Takara Shuzo Co., Ltd.), pUC118 (a product of Takara Shuzo Co., Ltd.) and pPA1 (Japanese Published Unexamined Patent Application No. 233798/88).

The paragraph at page 38, lines 9-23 have been amended as follows:

That is, N-acetylglucosamine monosaccharides, oligosaccharides having N-acetylglucosamine residue at the non-reducing termini thereof, or complex carbohydrates having N-acetylglucosamine residue at the non-reducing termini of sugar chains thereof,

are used as the acceptor substrate while the polypeptide of the present invention obtained in the method described in item (2) above is used as the enzyme source, and reaction products having galactose transferred via β1,3-linkage to N-acetylglucosamine monosaccharide or N-acetylglucosamine residue of the acceptor substrate can be produced by allowing said receptor substrate, said enzyme source and uridine-5'-diphosphate galactose (UDP-Gal) to be present in an aqueous medium to thereby form and accumulate said reaction products in said aqueous medium and collecting said reaction products from said aqueous medium.

The paragraph starting at page 44, line 36 and ending at page 45, line 7 has been amended as follows.

The class and subclass of the antibody [is] are determined using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. The class of the antibody means isotype of the antibody, and for example, mention can be made of IgG, IgA, IgM,IgD and IgE in human. The subclass of the antibody means isotype in the class, and for example, mention can be made of IgG1, IgG2a, IgG2b and IgG3 in mouse, and IgG1, IgG2, IgG3 and IgG4 in human.

The paragraph starting at page 54, line 29 and ending at page 55, line 4 has been amended as follows.

Fig. 1, A shows the results of measurement of the expression levels of type 1 sugar chains (sialyl-Lewis a sugar chain, Lewis a sugar chain, Lewis b sugar chain) in a

wide variety of human cancer cell lines. Each kind of cell was stained with a fluorescent antibody using anti-sialyl-Lewis a sugar chain antibody (19-9), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody [(Neokokusai)] (TT42) and then analyzed by FACS. The reactivity with each antibody is shown as +++, ++, +, ±, and –, in the order of decreasing reactivity. – means the absence of reactivity with the antibody. NT means that the analysis was not conducted.

The paragraphs at page 55, lines 15-32 have amended as follows.

Fig. 3, A shows the results of indirect fluorescent antibody staining with anti-sialyl-Lewis c sugar chain antibody (DU-PAN-2) and subsequent FACS analysis of Namalwa cells **[**[Namalwa (mock)]**]****(Namalwa-mock)** having the control plasmid (pAMo) introduced therein, or Namalwa cells (Namalwa-3GT5) having human β3Gal-T5 expression plasmid (pAMo-3GT5) introduced therein. The shaded histogram shows the result of analysis using A-PBS in place of DU-PAN-2.

Fig. 3, B shows the results of indirect fluorescent antibody staining with anti-sialyl-Lewis a sugar chain antibody (19-9), anti-sialyl-Lewis c sugar chain antibody (DU-PAN-2), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody ([Neokokusai] **TT42**) and subsequent FACS analysis of HCT-15 cells [[HCT15 (mock)]] **(HCT-mock)** having the control plasmid (pAMo) introduced therein, or HCT-15 cells (HCT-3GT5H) having human β3Gal-T5 expression plasmid (pAMo-3GT5) introduced

therein. The shaded histogram shows the result of analysis using A-PBS in place of DU-PAN-2.

The paragraph at page 56, lines 22-29 have been amended as follows:

Fig. 6, C shows the results of examination of the expression level of each isoform of human β3Gal-T5 cDNA in Colo205 cells by the RT-PCR method. After RT-PCR was conducted with the combination of primers shown in Fig. 6, A, the reaction product was cleaved with a restriction enzyme (XbaI or BsmI) shown in Fig. 6, to specify the isoform. **["none"]** (-) means that the restriction enzyme treatment was not conducted. The left line shows molecular markers (100 bp ladder).

The paragraph at page 58, lines 8-13 have been amended as follows:

The cells described above were cultured in a medium suitable for the respective cells, and then the cells were subjected to fluorescent antibody staining with anti-sialyl-Lewis a sugar chain antibody (19-9), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody (**[Neokokusai]** **TT42**) and analyzed by FACS.

The paragraph at page 60, lines 16-19 have been amended as follows:

As the internal controls, linear DNAs were prepared by cleaving plasmids prepared below (**[pBS]** **pUC119**-3GT1d, pBS-3GT2d, pBS-3GT3d, pBS-3GT4d) with suitable restriction enzymes to take the cDNA inserts.

The paragraph at page 60, lines 24-25 have been amended as follows:

By deleting a 183-bp sequence between StyI-StyI in human β3Gal-T3 cDNA in pBS-3GT3, pBS-3GT3d was prepared.

The paragraph at page 71, lines 5-10 have been amended as follows:

The single clone (HCT-3GT5H) thus obtained was subjected to indirect fluorescent antibody staining with anti-sialyl-Lewis a sugar chain antibody (19-9), anti-sialyl-Lewis c sugar chain antibody (DU-PAN-2, a product of Kyowa Medex), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody (**[Neokokusai] TT42**).

The paragraph at page 73, lines 19-21 have been mended as follows:

The respective cells ($1x10^7$ cells) were suspended in a solution [20 mmol/l HEPES (pH 7.2), 2% **[Triron] Triton** X-100] and sonicated in a short time to prepare a cell lysate.

The paragraph at page 76 , lines 1-3 have been amended as follows:

Said transformed cells were suspended in a solution [20 mmol/l HEPES (pH 7.2), 2% **[Triron] Triton** X-100] and sonicated in a short time to prepare a cell lysate solution.

The paragraph at page 76, lines 14-19 have been amended as follows:

Specifically, the activity was measured by identifying a product by high performance liquid chromatography (HPLC) after reaction at 37°C for 2 hours in 10 µl assay solution [14 mmol/l HEPES (pH 7.4), 75 µmol/l UDP-Gal (a product of SIGMA), 11 µmol/1 $MnCl_2$, 0.01% [Triron] **Triton** X-100, 25 µmol/l pyridylaminated sugar chain substrate, and the above cell lysate solution].

The paragraphs at page 79, lines 12-27 have been amended as follows:

The β3Gal-T5 transcripts in various human tissues (brain, lung, esophagus, stomach (body), stomach (antrum), jejunum, colon, liver, pancreas, spleen, kidney, adrenal, uterus, peripheral blood lymphocytes) were quantified by RT-PCR in the same manner as in item (7) in Example 2. The amount of the β3Gal-T5 gene transcript in each kind of organs is shown as a value relative to the amount (= 1000) of the β-actin transcript (Fig. 5).

It was revealed that the β3Gal-T5 transcripts are significantly expressed in the stomach (body), stomach (antrum), jejunum, colon and pancreas. Further, the β3Gal-T5 transcripts were slightly expressed in the brain, esophagus, kidney and uterus. On the other hand, the β3Gal-T5 transcripts were not expressed in the lung, liver, spleen, adrenal and peripheral blood lymphocytes.